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Effect of extracellular polymeric substances disintegration by ultrasonic pretreatment on waste activated sludge acidification

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ABSTRACT

In this study, batch tests were conducted to investigate the effect of extracellular polymeric substances (EPS) disintegration on anaerobic fermentation of waste activated sludge (WAS). Ultrasonic pretreatment was used to disintegrate EPS and accelerate WAS hydrolysis. The experimental results showed that more organic substances were released from sludge with increase of ultrasonic density, and the EPS were completely disintegrated at ultrasonic parameters beyond 2.0 W/mL and 15 min. The optimal ultrasonic density for short chain fatty acids (SCFAs) accumulation was 2.0 W/mL, and the SCFAs concentration reached 3166 mg/L after 5 days of anaerobic fermentation. The SCFAs mainly consisted of acetic and propionic acids, accounting for 88% of total SCFAs. Denaturing gradient gel electrophoresis (DGGE) analysis indicated that ultrasonic density significantly altered the bacterial communities by affecting the EPS disintegration degree. *Firmicutes*, *Proteobacteria* and *Bacteroidetes* were main species, contributing to proteins (PN) and polysaccharides (PS) degradation and SCFAs production.

Introduction

More than 30 million tons of waste activated sludge (WAS, water content 80%) is produced annually in China (Duan et al., 2012), and the treatment and disposal of WAS are the major issue in wastewater treatment plants (WWTPs). Recently, WAS has been used as renewable resource to produce various value-added products, such as short chain fatty acids (SCFAs), biofuel, biopesticides or biosolids (Alam et al., 2003; Yezza et al., 2006; Liu et al., 2014; Rivero et al., 2014). Among them, the SCFAs have showed a great potential as a low cost internal carbon source for biological nutrient removal processes (Tong and Chen, 2009), in which both sludge recycling and high nutrient removal were accomplished at the same time (Gao et al., 2011).

Anaerobic fermentation is recognized as an efficient process to obtain SCFAs from WAS (Cokgor et al., 2009). Mass reduction, SCFAs production, and improved dewaterability of sludge are the most important advantages of anaerobic fermentation. The disadvantage of anaerobic fermentation is the slow degradation rate of biomass, due to the inhibition of cell wall and membrane of bacteria (Ge et al., 2010). Sludge hydrolysis has been considered as the rate-limiting step of anaerobic fermentation, and the extracellular polymeric substances (EPS) disintegration was the key factor of sludge hydrolysis (Bougrier et al., 2005). In an effort to accelerate the rate of sludge hydrolysis, various pretreatment methods have been developed, such as ultrasonic (Kang et al., 2011), alkaline (Chen et al., 2007), and biological treatment (Oh et al., 2014). The employment of ultrasonic pretreatment is generally regarded as an effective way to destroy EPS and/or cells and is nonhazardous to the environment (Zhang et al., 2007); concomitantly, the SCFAs production can be enhanced, and the fermentation time can be shortened by soluble organic substrates released from WAS (Nakasaki et al., 2009).

Anaerobic fermentation is a complex biochemical process, which requires the synergic action of a variety of distinct bacteria (Park et al., 2013). The species and functions of bacteria in anaerobic fermentation process can be changed by many factors such as pH, oxidation reduction potential (ORP), and substrate characteristics.

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It was reported that organic matters released from ultrasonic sludge as the key substrates for SCFAs production altered the bacterial community (Kang et al., 2011). A deeper investigation on bacterial community structure and its dynamics can provide invaluable information for controlling the anaerobic fermentation process, and improving SCFAs production. The molecular biotechnology has provided a valuable tool for the examination of the complex bacterial communities in sludge fermentation process (Zheng et al., 2013). The species and quantities of microbes and their functions have been well documented.

In this study, ultrasonic pretreatment was used to destroy sludge flocs, and the disintegration process was evaluated by soluble proteins (PN) and polysaccharides (PS) concentrations. In the anaerobic fermentation tests, the effects of EPS disintegration on PN and PS solubilization, and SCFAs production were investigated. Of interest, the composition of SCFAs was examined, and the mechanisms of SCFAs production were discussed. The effects of EPS disintegration on bacterial communities were analyzed by polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis (DGGE).

Materials and methods

Experimental materials

The WAS used in this study was collected from the secondary sedimentation tank of Wenchang municipal wastewater treatment plant in Harbin, China. The sludge was settled at 4 °C for 12 h. The detailed characteristics of concentrated sludge are summarized in Table 1. After heat extraction of WAS, the soluble PN and PS concentrations in extracted EPS were 1395 and 349 mg/L, respectively.

The anaerobic sludge was collected from an upflow anaerobic sludge blanket (UASB) reactor in our laboratory. The VSS was 15,000 ± 500 mg/L.

Experimental design

Sludge was pretreated in an ultrasonicator (JY98-IIIN, Xinzhi Inc., China) at a frequency of 20 kHz, and the ultrasonic densities and times were set in range of 0–3.0 W/mL and 0–15 min, respectively. Then the ultrasonic sludge (0, 1.0, 2.0 and 3.0 W/mL, 15 min) were used for anaerobic fermentation. The fermentation experiment was carried out in four identical anaerobic reactors with working volume of 2 L, and each reactor contained 800 mL ultrasonic sludge and 800 mL anaerobic sludge. Then, the reactors were continuously operated 15 days at room temperature, and the stirred speed was controlled at 100 rpm. The pH values of all reactors were maintained at 9 by automatically adding 2 M sodium hydroxide or 2 M hydrochloric acid to restrain SCFAs consumption by methanogens.

Table 1
Characteristics of waste activated sludge.

Item	Value
pH	6.5–7.0
Total suspended solid (TSS) (mg/L)	18,500 ± 250
Volatile suspended solid (VSS) (mg/L)	12,100 ± 150
Total chemical oxygen demand (TCOD) (mg/L)	18,200 ± 200
Soluble COD (SCOD) (mg/L)	190 ± 30
Total PN (mg/L)	10,700 ± 50
Soluble PN (mg/L)	93
Total PS (mg/L)	1950 ± 20
Soluble PS (mg/L)	25
Particle size (μm)	160 ± 25

Analytical methods

TCOD, SCOD, TSS and VSS were measured according to Standard Methods (APHA, 2005). The average particle size of sludge was detected by particle size analyzer (S3500, Microtrac, USA). All sludge samples were centrifuged at 10,000 rpm for 5 min, and the supernatant was filtrated with 0.45 μm cellulose acetate membranes. The filtrate was immediately analyzed for PN, PS and SCFAs. The PN content was quantified according to Lowry method with bovine serum albumin as the standard (FrØlund et al., 1995), and the PS concentration was determined on the basis of the phenol-sulfate acid method with glucose as the standard (Herbert et al., 1971). The composition of SCFAs was analyzed by gas chromatography (Agilent 6890 N) using a flame ionization detector (FID) and DB-WAXETR column (30 m × 0.53 mm × 1.0 μm) (Chen et al., 2007), and the total SCFAs was recorded as the sum of acetic, propionic, n-butyric, iso-butyric, n-valeric and iso-valeric acids. All the experiments were conducted in triplicate with average and standard deviation reported.

EPS extraction

The sludge EPS were extracted using heat extraction method according to the previous research (Li and Yang, 2007). Briefly, 25 mL sludge was first washed three times with deionized water, and then centrifuged in a 50 mL tube at 4000 rpm for 5 min to remove the supernatant. After that, the cell pellet was re-suspended with 0.05% NaCl solution and heated at 80 °C for 30 min, and then centrifuged at 10,000 rpm for 10 min. The PN and PS in supernatant were regarded as the EPS of sludge.

DNA extraction

Total DNA was extracted from biomass of each anaerobic reactor on the 15th day using the E.Z.N.A Soil DNA Kit (Omega Bio-Tek. Inc., Georgia, USA). Samples were centrifuged at 10,000 rpm for 5 min before extraction. Each extraction was performed in duplicate and the extracted DNA was eluted in Tris–HCl buffer (pH 8.0) and stored at −20 °C.

PCR and DGGE

The V3 region was amplified by PCR using the forward primer 338f (5′-ACTCCTACGGGAGGCAGCAG-3′) and the reverse primer 534r (5′-ATACCGCGGCTGCTGG-3′) with a GC clamp (5′-CGCCCGCCGCGCCCCGCGCCCGGCCCGCCGCCCCCGCCCC-3′) at the 5′ end. The detailed steps of PCR amplification followed the literature (Shin et al., 2010a). DGGE was performed with the DCode Universal Mutation Detection system (Bio-Rad, USA). Denaturing gradient used in this experiment ranged from 40% to 60%. Electrophoresis was carried out at a constant voltage of 140 V for 7 h. After electrophoresis, the gel was stained with ethidium bromide and then scanned under UV transillumination (Kim et al., 2011).

Cloning of 16S rRNA fragments

For further sequencing and phylogenetic analysis, bands of interest were excised from the gels. Each gel fragment was crushed, and the DNA was resolved in 50 μL of sterile deionized water at 4 °C. PCR was performed with the primer 338f and 534r without a GC clamp. The PCR fragments were purified using E.Z.N.A Cycle Pure Kit (Omega Bio-Tek. Inc., Georgia, USA) and cloned in *Escherichia coli DH5a* (Takara, Dalian, China) using PMD19-T Vector (Takara, Dalian, China) according to the manufacturer's instruction.